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Physiological changes in broiler chickens subjected to dietary ajwain (*trachyspermum ammi l.*) essential oil in encapsulated and conventional forms within a wheat-based diet

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ABSTRACT

The aim of this study was to investigate the effects of different diet types, forms, and contents of ajwain essential oil (AEO) on various physiological characteristics of broiler chickens, including cell-mediated immunity responses, intestinal morphology, and microflora. A total of 1500 one-day-old male broiler chickens were allocated to different treatments based on a 2 × 3 × 2 factorial arrangement, considering diet types (corn and corn-wheat), contents of AEO (0, 150, and 300 mg/kg of diet), and forms of AEO (conventional and encapsulated). The results indicated that the broiler chickens fed the diet containing 150 ppm EO demonstrated reduced skin thickness in response to a 2,4-dinitrochlorobenzene challenge, 24 h after injection, compared to those receiving a diet without EO ($P < 0.05$). Increasing the EO content led to an increase in the villous height to crypt depth ratio in the jejunum of broiler chickens receiving 300 ppm EO ($P < 0.05$). Moreover, there was a slight improvement in the villous height to crypt depth ratio in the jejunum of broiler chickens fed the corn-wheat diet ($P = 0.07$). Broiler chickens fed the 300 ppm EO showed a lower total bacterial population compared to those fed the 150 ppm EO ($P < 0.05$). Finally, the use of EO at a content of 150 ppm improved cellular immune response, while EO at a content of 300 ppm improved the morphology and overall population of intestinal bacteria. Furthermore, the inclusion of wheat-corn diets exhibited enhanced morphological characteristics of the intestines. However, the forms of AEO did not exert any significant influence on the physiological traits.

1. Introduction

Wheat is a viable substitute for corn in the diet of broiler chickens due to its high nutrient content, including essential amino acids and proteins. However, its inclusion is constrained by the presence of non-starch polysaccharides (NSPs), which can increase the viscosity of the digesta in the small intestine. This can lead to slower food passage and create a favorable environment for the proliferation of harmful bacteria such as *Escherichia coli* (Collier et al., 2003). It has been observed that the binding of soluble NSPs to intestinal enzymes can decrease their activity, resulting in reduced chicken performance. Nonetheless, the supplementation of herbal plants in the diet has been shown to enhance the activity of pancreatic and intestinal enzymes, leading to improved digestion speed (Sarica et al., 2005). It has been demonstrated that essential oils (EOs), particularly those derived from plants, can enhance the activity of trypsin and amylase enzymes. Plant-based EOs contain active compounds that stimulate the activity of digestive enzymes in the mucosa of the intestines and pancreas. Studies have shown that the combination of carvacrol and thymol can effectively enhance the performance, intestinal microbial population, and morphology of broiler chickens that are fed a wheat-based diet (Hashemipour et al., 2016; Mahmoodi Bardzardi et al., 2014a, b; Mohammadi et al., 2014).

Plant compounds, have the ability to inhibit the growth and spread of both pathogenic and non-pathogenic species in the intestines (Sarica et al., 2005). Ajwain (*Trachyspermum ammi*) is an herbaceous plant belonging to the *Apiaceae* family and is commonly known as *Carum copticum*. The essential oil derived from ajwain, known as ajwain essential oil (AEO), is highly valued for its antibacterial properties and is

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often recommended for such purposes. Usually, thymol is the primary constituent of ajwain essential oil, comprising approximately 35 % to 60 % of its composition. The non-thymol fraction, known as thymene, contains compounds such as para-cymene, γ-terpinene, α-pinene, β-pinene, α-terpinene, styrene, delta-3-carene, β-phellandrene, terpinene-4-ol, and carvacrol. Additionally, oleic acid, linoleic acid, palmitic acid, petroselinic acid, and resin acids have been isolated from ajwain fruits (Zarshenas et al., 2014).

Nanotechnology has been explored as a means to enhance the availability of EOs in the livestock and poultry industry by increasing their surface area. The encapsulation of plant EOs within polymer coatings has been extensively researched for the purpose of protecting these compounds from environmental conditions and enhancing their antimicrobial properties (Hosseini & Meimandipour, 2018; Meimandipour et al., 2017). Although studies have investigated the antibacterial properties of nanoparticles containing EOs against various bacterial strains (Esmaeili & Rafiee, 2014; Raphael & Meimandipour, 2017), limited information is available regarding the effects of encapsulated ajwain essential oil (E-AEO) on broiler chickens. E-AEO may provide increased stability and improved absorption in animals, potentially leading to a more effective form of EO. The objective of this study was to investigate the impacts of different levels of E-AEO in nanoparticle and AEO forms on cell-mediated immunity, intestinal microbiology, and morphology in broiler chickens fed diets based on corn and wheat.

2. Materials and methods

All the experimental procedures used in this study were approved by the Animal Ethics Committee of the Animal Science Research Institute of Iran and the Animal Care Committee of the Department of Animal Science, University of Tehran. The AEO utilized in the study was obtained from the traditional medicine department of the Barij Essence Pharmaceutical Company (Kashan, Iran). Before incorporating AEO into the diets, its composition was analyzed using gas chromatography (Agilent 6890N, Agilent Technologies, Paris, France) interfaced with mass spectroscopy (Agilent 5973N, Agilent Technologies). The results of the GC–MS analysis of AEO are presented in Table 1. The encapsulation of AEO in nanoparticles was conducted by the National Institute of Genetic Engineering and Biotechnology (Tehran, Iran) as described by Hosseini and Meimandipour (2018).

2.1. Experimental design, broiler chicken and diets

A total of 1500 one-day-old male broiler chickens (Ross308) were obtained from a local hatchery. They were weighed and randomly divided into 60 floor pens (3 m^2), and covered with fresh wood shavings. The broiler chickens were assigned to twelve treatments, with five replicates per treatment. Each replicate consisted of 25 broiler chickens. The experimental design followed a completely randomized design with a factorial arrangement of 2 × 3 × 2. The factors included two types of diet (corn, corn-wheat), three levels of AEO content (0, 150, and 300 mg/kg of diet), and two forms of AEO (conventional and encapsulated). The AEO was mixed with a carrier (soybean oil) and added to the basal diet. The broiler chickens were vaccinated for infectious bronchitis (Nobilis IB 4/91; Merck Animal Health, Boxmeer, the Netherlands) on the first day, avian influenza (Nobilis Infuenza TRT; Merck Animal Health) on day 7 and Newcastle disease (Nobilis *G* + ND; Merck Animal Health) on days 18 and 28. The feeding regimen for the broiler chickens consisted of three different diets: starter (day 0 to 10), grower (day 11 to 24), and finisher (day 25 to 42). The basal diet was carefully formulated to meet the nutrient requirements of the broiler chickens as recommended by Ross 308 broiler chicken management guide (Table 2). The broiler chickens had *Ad libitum* access to both feed and water throughout the study. The temperature and humidity levels were carefully regulated and maintained within the optimal range for the birds' well-being. During the initial three days, the broiler chickens were exposed to a constant 24 h light period. From day 4 onwards, the photoperiod was adjusted to 23 h of light and 1 h of darkness per day until day 42. Throughout the study, the ventilation rate in the chicken house remained consistent. Additionally, the initial house temperature of 32 °C was gradually reduced to reach 20 °C by day 42. Freshly prepared diets in mash form were provided on a weekly basis.

Table 1
Composition of ajwain essential oil.[1]

Major components	(%)
Thymol	41
γ-Terpinene	30
Ppara-cymene	16
Myrcene	5
β-Phellandrene	5
β-Pinene	1

[1] Identification by gas chromatography coupled to mass spectroscopy, National Institute of Standards and Technology (Gaithersburg, MD, US).

2.2. Cell-mediated immunity

In the 42nd experiment, 10 broiler chickens from each treatment were identified with distinct colors and sensitized by administering 0.25 mL of a solution containing 2,4-dinitrochlorobenzene (DNCB) at a concentration of 10 mg/mL in acetone. The DNCB used in this study was obtained from SIGMA Aldrich® (2,4-Dinitrochlorobenzene, SKU 237,329). The resulting reaction was evaluated by measuring the increase in skin thickness using electronic digital calipers (Forbes Gokak,

Table 2
Composition of the experimental diets for broiler chickens (as-fed basis).

Item	Starter (d 0 to 10)		Grower (d 11 to 24)		Finisher (d 25 to 42)	
Ingredients (%)						
Corn	51.5	55.7	43.1	57.2	40.1	63.3
Wheat	5.00	0.00	15.0	0.00	25.0	0.00
Soybean meal (44 % CP[a])	38.1	38.9	35.8	37.0	28.6	31.0
Soybean oil	1.30	1.30	2.30	2.00	2.40	1.80
Calcium carbonate	1.10	1.10	1.20	1.20	1.15	1.15
Dicalcium phosphate	1.90	1.90	1.68	1.68	1.83	1.83
Common salt	0.25	0.25	0.20	0.20	0.20	0.20
DL- methionine	0.27	0.27	0.19	0.19	0.17	0.17
L- lysine HCL	0.08	0.08	0.06	0.06	0.05	0.05
Vitamin premix[b]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[c]	0.25	0.25	0.25	0.25	0.25	0.25
Chemical composition						
Metabolizable energy (MJ/kg)	12.14	12.14	12.39	12.39	12.56	12.56
CP (%)	22.0	22.0	21.3	21.3	19.1	19.1
Ca (%)	0.90	0.90	0.87	0.87	0.85	0.85
Available P (%)	0.45	0.45	0.43	0.43	0.40	0.40
Na (%)	0.19	0.19	0.16	0.16	0.16	0.16
Cl (%)	0.28	0.28	0.24	0.24	0.23	0.23
Lys (%)	1.27	1.27	1.19	1.19	1.00	1.00
Met (%)	0.60	0.60	0.54	0.54	0.47	0.47
Met + Cys (%)	0.91	0.91	0.84	0.84	0.75	0.75
Thr (%)	0.81	0.81	0.77	0.77	0.68	0.68
Arg (%)	1.45	1.45	1.40	1.40	1.23	1.23
Anion-cation balance (mEq /kg)	245	245	235	235	214	214

[a] CP = crude protein.
[b] Supplying per kilogram of diet: all-*trans* retinol acetate, 1.32 mg; cholecalciferol, 0.05 mg; menadione sodium bisulphate, 1.2 mg; thiamin mononitrate, 0.8 mg; riboflavin, 2.2 mg; pyridoxine, 0.8 mg; cyanocobalamin, 0.06 mg; calcium pantothenate, 12.1 mg; niacin, 20 mg; DL-α-tocopheryl acetate, 15 mg; folic acid, 1 mg; and choline chloride, 160 mg.
[c] Supplying per kilogram of diet: Fe ($FeSO_4$), 50 mg; I (Calcium iodate), 1 mg; Se (Sodium selenite), 0.2 mg; Cu ($CuSO_4$), 10 mg; Zn (ZnO), 66 mg, and Mn (MnO_2), 100 mg.

Mumbai, India) at three different time points: before the challenge, as well as 24 and 48 h after the challenge, following the methodology established by Verma et al. (2004).

Additionally, in the same experiment, 10 broiler chickens from each treatment were marked with colors and intradermally injected with phytohemagglutinin (PHA) in the skin between their third and fourth digits of the right foot, using a volume of 0.1 mL. As a control, the left foot of each broiler chicken was injected with 0.1 mL of sterile physiological saline solution. The cellular response triggered by PHA, which represents the immune response mediated by cells, was assessed at 24 and 48 h after the PHA challenge by measuring the skin thickness using an electronic caliper with a precision of 0.01 mm (Electronic Digital Caliper) (Corrier & Deloach, 1990).

2.3. Gut morphology

In the 42nd experiment, 10 broiler chickens from each treatment were euthanized by cervical dislocation. Their abdominal cavities were opened, and the entire digestive tract was immediately extracted. Subsequently, 2 cm segments of the jejunum, specifically the midpoint of the

Table 3
Effects of diet type, contents and forms of ajwain essential oil on jejunum morphology of broiler chickens and cell-mediated immunity of broiler chickens at 42 days of age.[a,b]

			Jejunum morphology (mm)				Cell-mediated immunity (24 and 48 h after injection)(mm)			
Diet type	AEO content(mg/kg)	AEO form	Villous height	Villous width	Crypt depth	Vh/Cd	DNCB (24 h)	DNCB (48 h)	PHA (24 h)	PHA (48 h)
Diet type x AEO content x AEO form										
Corn	0	AEO	1.15	0.125	0.055	23.56	1.41	0.36	1.63	1.18
	0	E-AEO	1.14	0.124	0.055	23.55	1.40	0.35	1.63	1.17
	150	AEO	1.31	0.118	0.055	25.10	1.25	0.37	1.64	1.33
	150	E-AEO	1.32	0.120	0.050	27.28	1.19	0.52	1.54	1.17
	300	AEO	1.28	0.125	0.045	33.56	1.39	0.48	1.63	1.14
	300	E-AEO	1.28	0.118	0.053	27.53	1.51	0.49	1.49	1.13
Corn – Wheat	0	AEO	1.37	0.125	0.048	29.68	1.63	0.55	1.45	1.31
	0	E-AEO	1.37	0.125	0.048	29.68	1.62	0.55	1.44	1.30
	150	AEO	1.29	0.130	0.045	30.29	1.42	0.60	1.55	1.27
	150	E-AEO	1.29	0.160	0.055	23.64	1.27	0.38	1.46	1.14
	300	AEO	1.24	0.133	0.35	44.91	1.37	0.56	1.55	1.31
	300	E-AEO	1.36	0.148	0.40	40.98	1.33	0.53	1.81	1.30
SEM			0.05	0.007	0.008	6.10	0.10	0.11	0.10	0.10
AEO content x AEO form										
	0	AEO	1.26	0.125	0.051	26.62	1.52	0.45	1.54	1.24
	150	E-AEO	1.26	0.125	0.051	26.62	1.50	0.46	1.53	1.22
	300	AEO	1.30	0.124	0.050	27.69	1.34	0.48	1.60	1.30
	0	E-AEO	1.31	0.140	0.053	25.46	1.23	0.45	1.50	1.15
	150	AEO	1.26	0.129	0.040	39.23	1.38	0.52	1.59	1.22
	300	E-AEO	1.32	0.132	0.046	34.26	1.42	0.51	1.65	1.21
SEM			0.04	0.005	0.005	4.31	0.07	0.08	0.07	0.07
Diet type x AEO form										
Corn		AEO	1.25	0.123	0.052	27.40	1.35	0.40	1.63	1.21
		E-AEO	1.25	0.121	0.053	26.12	1.37	0.46	1.55	1.16
Corn – Wheat		AEO	1.30	0.129	0.043	34.96	1.47	0.57	1.52	1.30
		E-AEO	1.34	0.144	0.048	31.44	1.40	0.49	1.57	1.25
SEM			0.03	0.004	0.004	3.52	0.06	0.06	0.06	0.05
Diet type x AEO content										
Corn	0		1.15[b]	0.125[ab]	0.055	23.56	1.40	0.35	1.63	1.18
	150		1.32[a]	0.119[b]	0.053	26.19	1.22	0.44	1.59	1.25
	300		1.28[ab]	0.121[ab]	0.049	30.54	1.45	0.49	1.56	1.13
Corn – Wheat	0		1.37[a]	0.125[ab]	0.048	29.68	1.63	0.55	1.45	1.31
	150		1.29[ab]	0.145[a]	0.050	26.96	1.34	0.49	1.50	1.21
	300		1.30[ab]	0.140[ab]	0.038	42.94	1.35	0.54	1.68	1.30
SEM			0.04	0.005	0.005	4.31	0.07	0.08	0.07	0.07
Diet type	Corn		1.25[b]	0.122[b]	0.052	26.76	1.36	0.43	1.59	1.18
	Corn-Wheat		1.32[a]	0.137[a]	0.045	33.20	1.44	0.53	1.54	1.27
SEM			0.02	0.003	0.003	2.49	0.04	0.05	0.04	0.04
AEO content	0		1.26	0.125	0.051	26.62[b]	1.52[a]	0.45	1.54	1.24
(mg/kg)	150		1.30	0.132	0.051	26.58[b]	1.28[b]	0.46	1.55	1.23
	300		1.29	0.131	0.043	36.75[a]	1.40[ab]	0.51	1.62	1.22
SEM			0.02	0.003	0.004	3.05	0.05	0.06	0.05	0.05
AEO form	AEO		1.27	0.126	0.047	31.18	1.41	0.48	1.57	1.25
	E-AEO		1.29	0.133	0.050	28.78	1.39	0.47	1.56	1.20
SEM			0.02	0.003	0.003	2.49	0.04	0.05	0.04	0.04
P-value										
Diet type			0.04	0.001	0.14	0.07	0.16	0.13	0.39	0.12
AEO content			0.52	0.41	0.27	0.03	0.006	0.72	0.43	0.92
AEO form			0.55	0.14	0.53	0.49	0.69	0.84	0.82	0.35
Diet type x AEO content			0.01	0.05	0.74	0.41	0.07	0.58	0.09	0.26
Diet type x AEO form			0.63	0.07	0.65	0.75	0.43	0.29	0.24	0.94
AEO content x AEO form			0.72	0.30	0.86	0.84	0.56	0.97	0.49	0.49
Diet type x AEO content x AEO form			0.73	0.41	0.71	0.79	0.83	0.45	0.28	0.99

[a,b] Means in each column with different superscripts are different ($P < 0.05$). AEO = ajwain essential oil, E-AEO = Encapsulated ajwain essential oil, and SEM = pooled standard error of the mean, Vh/Cd: Villous height to Crypt depth ratio, DNCB = Dinitrochlorobenzene, PHA = Phytohemagglutinin.
[b] Data represent the means of 5 cages ($n = 5$).

jejunum, were promptly dissected for morphological measurements of the intestinal tract. The jejunum samples were then preserved in 10 % formaldehyde, fixed in bouin's solution, and embedded in paraffin. Histological examinations were conducted following the methodology described by Iji et al. (2001). Thin sections with a thickness of 6 μm were prepared from each sample, stained with hematoxylin and eosin, and observed under light microscopy. Villus height, villus width, and crypt depth were measured using a linear scaled graticule.

2.4. Gut microflora

At the 42nd experiment, 10 broiler chickens from each treatment were euthanized by cervical dislocation. The contents of the ileum were collected under sterile conditions and transferred to a sterile plastic bag. Subsequently, the samples were sent to the microbiology laboratory in Tehran, Iran. The samples were diluted with phosphate buffer solution at a ratio of 1:10 and mixed for two minutes using a stomacher (80I, England). After dilution, 100 μl of each sample was plated onto specific media for different types of microorganisms. MacConkey agar (Merck, Darmstadt, Germany) was used to detect *enterobacter* and *coliform* bacteria, MRS agar (Merck, Darmstadt, Germany) was used for *lactobacilli spp,* plate count agar was used for total aerobes, and sabouraud dextrose agar was used for yeast and fungi. All plates were then incubated at 37 °C for 24 to 48 h. The results were reported as the logarithm of colony forming units (CFU) per gram of caecum content as log10 cfu/g (Yang et al., 2012).

2.5. Statistical analysis

The data was analyzed using the general linear models procedure of SAS, 2003. The replicate cage was considered as the experimental unit. The model consisted of the main effects (type of diet, AEO contents, and AEO forms) as well as their interactions. The experimental design followed a completely randomized design with a factorial arrangement of $2 \times 3 \times 2$. To assess significant differences among the means, Tukey's multiple-range test was used at a significance level of $P < 0.05$.

3. Results and discussion

3.1. Cell-mediated immunity

The interaction between diet types and EO contents and forms did not have a significant effect on cell-mediated immunity, specifically DNCB and PHA responses, in the skin of broiler chickens (Table 3). However, broiler chickens that were fed a diet containing 150 ppm EO exhibited a lower skin thickness (mm) after being injected with DNCB compared to broiler chickens that did not receive EO in their diet ($P < 0.05$). Furthermore, there was a slight improvement in the skin thickness (mm) of broiler chickens fed a corn-based diet containing 150 ppm EO 24 h after DNCB injection, although the significance level was marginal ($P = 0.07$) (Table 3). The AEO in encapsulated and conventional forms did not have a significant effect on DNCB and PHA responses in the skin of broiler chickens.

Cell-mediated immunity refers to a type of immune response that involves the activation of T-lymphocytes, also known as T cells. T cells play a crucial role in recognizing and eliminating specific pathogens, infected cells, and tumor cells within the body. In a study, the effects of PHA on cell-mediated immunity were investigated. PHA is a plant lectin derived from red kidney beans and acts as a mitogen, stimulating the proliferation of T cells. Researchers utilized PHA as a challenge agent to evaluate the immune response. By measuring the ability of T cells to divide and proliferate in response to the mitogen, the overall functionality and responsiveness of T cells were assessed. These findings support the widespread use of the PHA-skin test as a reliable method to evaluate acquired T-cell-mediated immunocompetence in various biological disciplines (Tella et al., 2008). However, in the present study, the experimental treatments did not demonstrate an effect on cell-mediated immunity (PHA) in the skin of broiler chickens.

Dinitrochlorobenzene (DNCB) is a chemical compound that forms complexes with proteins in the skin. This compound acts as an immune modulator by influencing the activity of different cells involved in the immune response, including langerhans cells, dendritic cells, and macrophages. Moreover, DNCB plays a role in the activation of T cells, which are crucial for the immune system's functioning (Zhang et al., 2009). Previous research by Mathivanan and Kalaiarasi (2007) has demonstrated that the inclusion of probiotics, drugs, and plant EOs in the diet can enhance cellular immune responses, which aligns with the findings of the current study. However, exposure to DNCB can lead to contact hypersensitivity in individuals, triggering an inflammatory response through the activation of T cells. In this study, broiler chickens that were fed a corn-based diet containing 150 ppm EO exhibited a slight improvement in skin thickness 24 h after being injected with DNCB. The T cells were exposed to DNCB, resulting in various immune responses such as cytokine production, T cell proliferation, and migration (Jegal et al., 2020). Jegal et al. (2020) were investigated the molecular mechanisms and signaling pathways involved in cell-mediated immunity using DNCB and PHA. By assessing the activation and proliferation of T cells, which play a crucial role in mounting immune responses against pathogens, diseases, and other threats, they gained valuable insights into the immune system's ability to recognize and respond to foreign antigens, allergens, and tumor cells. The study of cell-mediated immunity using challenge agents like DNCB and PHA provides important knowledge for the development of targeted therapies and vaccines aimed at specific T cell responses (Jegal et al., 2020).

3.2. Gut morphology

The study findings revealed that the interaction between of diet types and EO contents/forms did not have an impact on the crypt depth and the ratio of villous height to crypt depth in the jejunum of broiler chickens (Table 3). However, broiler chickens fed a diet containing corn-wheat without EO or corn + 150 ppm EO exhibited greater villous height compared to those receiving a diet containing corn without EO ($P < 0.01$). In the diet containing corn + 150 ppm EO, the width of the villous was reduced compared to the diet containing corn-wheat + 150 ppm EO ($P < 0.05$). Broiler chickens fed the corn-wheat diet displayed greater villous height and width compared to those fed the corn diet ($P < 0.01$). Moreover, an increasing in EO content resulted in a significant increase in the ratio of villous height to crypt depth in the jejunum of broiler chickens receiving 300 ppm EO ($P < 0.05$). Additionally, there was a slight improvement in the ratio of villous height to crypt depth in the jejunum of broiler chickens fed the corn-wheat diet ($P = 0.07$) (Table 3). The AEO in encapsulated and conventional forms did not have a significant effect on gut morphology in the jejunum of broiler chickens.

Intestinal morphological characteristics, including villus height, crypt depth, and the villus height to crypt depth ratio, serve as reliable indicators of intestinal health and are closely related to the mucous membrane's absorptive capacity (Attia et al., 2019). The villi in the intestines play a key role in nutrient absorption, and their height reflects the mucosa's ability to efficiently absorb nutrients. Crypts, on the other hand, are responsible for the generation of new epithelial cells, and their depth indicates the rate of tissue turnover necessary for the renewal of villi. The villus height to crypt depth ratio is a valuable parameter for evaluating the absorptive capacity of the small intestine (Shang et al., 2020). In this study, it was observed that broiler chickens fed corn-wheat diets exhibited an increase in villus height in the jejunum, indicating a larger surface area for improved nutrient absorption. Additionally, the inclusion of a corn-wheat supplement resulted in an increased ratio of villus height to crypt depth in the jejunum, indicating a further enhancement in the intestinal absorptive capacity. These findings can be attributed to the positive effects of dietary fiber on gut microbiota composition, intestinal morphology, and associated metabolites (Shang

et al., 2020). Similarly, Rezaei et al. (2011) also reported positive effects of insoluble fiber on the ratio of villus height to crypt depth in the ileum.

In a study conducted by Kolbadinejad and Rezaeipour (2020), it was observed that broiler chickens fed a diet supplemented with ajwain seed exhibited longer villus length. This indicates that the addition of ajwain seed to the diet has a positive effect on the development of villi in broiler chickens. Similarly, in a study by Hajiaghapour and Rezaeipour (2018), it was found that the width and height of the villi in the jejunum and ileum of quail breeders increased when their diet was supplemented with AEO, probiotics, and mannan-oligosaccharides. This suggests that including these substances in the diet has a positive impact on the growth and development of the villi in quail breeders. Furthermore, Pham et al. (2020) demonstrated that broiler chickens co-challenged with *Eimeria* spp./*C. perfringens* showed greater villus height and villus height/crypt depth ratio when their diet was supplemented with a blend of encapsulated essential oils and organic acids. Also, Masouri et al. (2017) reported that the dietary supplementation of 500 mg/kg satureja khuzistanica essential oil resulted in increased villus height and villus height-to-crypt depth ratios, along with a decreased crypt depth of the duodenum compared to the control diet. Ghazanfari et al. (2015) conducted a study where birds fed antibiotic and coriander essential oil exhibited higher villus height and crypt depth compared to the control group. The supplementation of coriander essential oil significantly reduced the thickness of the small intestinal epithelium compared to the

Table 4
Effects of diet type, contents and forms of ajwain essential oil on ileum microbiology of broiler chickens at 42 days of age (Results are expressed as log of colony forming units (CFU) per gram of ileum content (in log10 cfu/g)).[a,b]

Diet type	AEO content(mg/kg)	AEO form	Total bacteria	Lactobacilli	Yeast and fungi	Coliform	Enterobacter	Gram-negative bacteria
Diet type x AEO content x AEO form								
Corn	0	AEO	7.30	7.02	3.00	5.09	5.16	5.31
	0	E-AEO	7.25	7.10	2.95	5.13	5.13	5.25
	150	AEO	8.10	8.05	4.20	5.61	5.65	4.94
	150	E-AEO	8.42	8.28	4.26	5.37	5.36	5.16
	300	AEO	6.16	7.69	3.77	4.39	4.37	4.55
	300	E-AEO	7.28	8.14	3.49	5.05	5.19	5.59
Corn - Wheat	0	AEO	6.97	7.30	3.37	5.59	5.16	5.09
	0	E-AEO	6.88	7.35	3.40	5.49	5.10	5.14
	150	AEO	6.53	7.18	4.10	4.32	4.29	4.34
	150	E-AEO	8.54	8.36	3.93	5.44	5.85	5.47
	300	AEO	7.16	8.05	3.91	4.63	4.53	4.64
	300	E-AEO	6.10	8.42	4.43	5.57	5.23	5.80
SEM			0.59	0.68	0.68	0.71	0.71	0.63
AEO content x AEO form								
	0	AEO	7.13	7.16	3.18	5.34	5.16	5.20
	150	E-AEO	7.13	7.16	3.18	5.34	5.16	5.20
	300	AEO	7.32	7.61	4.15	4.96	4.97	4.64
	0	E-AEO	8.48	8.32	4.09	5.41	5.61	5.32
	150	AEO	6.66	7.87	3.84	4.51	4.45	4.59
	300	E-AEO	6.69	8.28	3.96	5.31	5.21	5.70
SEM			0.42	0.48	0.48	0.50	0.50	0.44
Diet type x AEO form								
Corn		AEO	7.19	7.58	3.65	5.03	5.06	4.93
		E-AEO	7.67	7.82	3.58	5.17	5.24	5.35
Corn - Wheat		AEO	6.89	7.51	3.79	4.85	4.66	4.69
		E-AEO	7.20	8.03	3.91	5.53	5.42	5.45
SEM			0.34	0.39	0.39	0.41	0.41	0.36
Diet type x AEO content								
Corn	0		7.30	7.02	3.00	5.09	5.16	5.31
	150		8.26	8.17	4.23	5.49	5.50	5.05
	300		6.72	7.92	3.63	4.72	4.78	5.07
Corn - Wheat	0		6.97	7.30	3.37	5.59	5.16	5.09
	150		7.53	7.77	4.01	4.88	5.07	4.91
	300		6.63	8.24	4.17	5.10	4.88	5.22
SEM			0.42	0.48	0.48	0.50	0.50	0.44
Diet type	Corn		7.43	7.70	3.62	5.10	5.15	5.14
	Corn - Wheat		7.04	7.77	3.85	5.19	5.04	5.07
SEM			0.24	0.28	0.28	0.29	0.29	0.25
AEO content	0		7.13[ab]	7.16	3.18	5.34	5.16	5.20
(mg/kg)	150		7.90[a]	7.97	4.12	5.18	5.29	4.98
	300		6.67[b]	8.09	3.90	4.91	4.83	5.15
SEM			0.29	0.34	0.34	0.36	0.36	0.31
AEO form	AEO		7.04	7.55	3.72	4.94	4.86	4.81
	E-AEO		7.43	7.92	3.75	5.35	5.33	5.40
SEM			0.24	0.28	0.28	0.29	0.29	0.25
P-value								
Diet type			0.27	0.86	0.56	0.82	0.79	0.84
AEO content			0.02	0.13	0.15	0.69	0.65	0.87
AEO form			0.26	0.35	0.95	0.32	0.26	0.11
Diet type x AEO content			0.75	0.71	0.71	0.49	0.85	0.90
Diet type x AEO form			0.81	0.72	0.81	0.51	0.49	0.64
AEO content x AEO form			0.31	0.76	0.98	0.73	0.72	0.47
Diet type x AEO content x AEO form			0.09	0.84	0.85	0.78	0.55	0.85

[a,b] Means in each column with different superscripts are different ($P < 0.05$). AEO = ajwain essential oil, E-AEO = Encapsulated ajwain essential oil, and SEM = pooled standard error of the mean.

[b] Data represent the means of 5 cages ($n = 5$).

control group. Moreover, in this study, an increase in the content of essential oil led to a significant increase in the ratio of villus height to crypt depth in the jejunum of broiler chickens receiving 300 ppm of essential oil.

3.3. Gut microflora

The interaction of different types of diets and various contents and forms of EO in the diet did not show any impact on the populations of total bacteria, *lactobacilli*, yeast and fungi, *coliform, enterobacter*, and gram-negative bacteria in the content of the ileum of broiler chickens (Table 4). However, broiler chickens that were fed a diet containing 300 ppm of EO exhibited a lower total bacterial population compared to those fed a diet containing 150 ppm of EO ($P < 0.05$). The AEO in encapsulated and conventional forms did not have a significant effect on gut microflora in the ileum of broiler chickens.

In a study conducted by Al-Kassie (2009), the addition of thyme and cinnamon to the diet of broiler chickens significantly reduced the total number of bacteria in the stomach, jejunum, and large intestine, which supports the findings of present research. It is believed that the lipophilic property and chemical structure of these additives may play a role in their antibacterial activity. Terpenoids and phenylpropanoids, due to their lipophilicity, have the ability to penetrate the bacterial membranes and reach the inner cellular components. Furthermore, the presence of functional groups and aromaticity in the structural properties of these additives also contribute to their antibacterial activity (Al-Kassie, 2009). It is widely recognized that most plant EOs exhibit slightly stronger antibacterial effects against gram-positive bacteria compared to gram-negative bacteria. Moreover, the population of *lactobacillus bacteria* increased up to a content of 100 ppm/kg of AEO, but decreased at a content of 150 ppm/kg (Burt, 2004). Pham et al. (2020) found that broiler chickens challenged with *C. perfringens* exhibited reduced bacterial counts in the intestine and lower gut lesion scores at 7 days after infection when they received a combination of encapsulated EOs and organic acids compared to those without the blend supplementation. Masouri et al. (2017) showed that the incorporation of 500 mg/kg satureja khuzistanica essential oil in the diet led to an increase in the population of *lactobacillus* in the cecum, while reducing the total bacterial and *E. coli* counts. However, the population of *lactobacillus* in the cecum decreased in broilers fed on the wheat-based diets.

An experiment was conducted to investigate the impact of different forms of wheat (airtight silo stored whole wheat, conventionally stored whole wheat, and ground wheat included in pellets) and the inclusion of xylanase in the diet on the production results and gastrointestinal characteristics of broiler chickens. The differences between the two types of whole wheat concerning the measured parameters were minimal, while significant variations were observed between birds fed pellets and those fed whole wheat. Feeding whole wheat led to lower levels of lactose-negative *enterobacteria* in the intestines and a tendency to reduce the populations of *clostridium perfringens* in the ileum and cecum (Engberg et al., 2004).

4. Conclusion

In summary, the findings of this study demonstrated that including 150 ppm of essential oil in the diet of broiler chickens resulted in thinner skin compared to the group without essential oil supplementation. Increasing the essential oil content to 300 ppm led to an increase in the ratio of villous height to crypt depth in the jejunum of the chickens. Furthermore, a diet consisting of a combination of corn and wheat slightly improved the villous height to crypt depth ratio. Moreover, broiler chickens fed a diet containing 300 ppm of essential oil exhibited a lower total bacterial population compared to those fed 150 ppm of essential oil. It was observed that the ajwain essential oil, regardless of its encapsulated or conventional form, did not have a significant impact on the physiological traits examined in this study.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

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